Research Communication

3,5-Diiodo-L-Thyronine Increases De Novo Lipogenesis in Liver from Hypothyroid Rats by SREBP-1 and ChREBP-Mediated Transcriptional Mechanisms

Antonio Gnoni[1]
Luisa Siculella[2]*
Giuseppina Paglialonga[2]
Fabrizio Damiano[2]* (ORCID)
Anna Maria Giudetti[2]

[1]Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari "Aldo Moro", 70124, Bari, Italy
[2]Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100, Lecce, Italy

Abstract

Hepatic de novo lipogenesis (DNL), the process by which carbohydrates are converted into lipids, is strictly controlled by nutritional and hormonal status. 3,5-Diiodo-L-thyronine (T2), a product of the 3,5,3′-triiodo-L-thyronine (T3) peripheral metabolism, has been shown to mimic some T3 effects on lipid metabolism by a short-term mechanism independent of protein synthesis. Here, we report that T2, administered for 1 week to hypothyroid rats, increases total fatty acid synthesis from acetate in isolated hepatocytes. Studies carried out on liver subcellular fractions demonstrated that T2 not only increases the activity and the expression of acetyl-CoA carboxylase and fatty acid synthase but also of other proteins linked to DNL such as the mitochondrial citrate carrier and the cytosolic ATP citrate lyase. Parallelly, T2 stimulates the activities of enzymes supplying cytosolic NADPH needed for the reductive steps of DNL. With respect to both euthyroid and hypothyroid rats, T2 administration decreases the hepatic mRNA level of SREBP-1, a transcription factor which represents a master regulator of DNL. However, when compared to hypothyroid rats T2 significantly increases, without bringing to the euthyroid value, the content of both mature (nSREBP-1), and precursor (pSREBP-1) forms of the SREBP-1 protein as well as their ratio. Moreover, T2 administration strongly augmented the nuclear content of ChREBP, another crucial transcription factor involved in the regulation of lipogenic genes. Based on these results, we can conclude that in the liver of hypothyroid rats the transcriptional activation by T2 of DNL genes could depend, at least in part, on SREBP-1- and ChREBP-dependent mechanisms. © 2019 IUBMB Life, 71(7):863–872, 2019

INTRODUCTION

Hepatic de novo lipogenesis (DNL) is the metabolic pathway by which excess of dietary carbohydrates is converted into fatty acids. DNL enzymes are regulated by transcriptional, posttranslational and allosteric mechanisms in response to nutrients and hormones (1–5). In addition, a coordinate action of mitochondrial and cytosolic DNL enzymes is required. Acetyl-CoA, the *primer* for the cytosolic synthesis of fatty acids, is produced in the

mitochondrial matrix mainly from glycolytic pyruvate by the action of pyruvate dehydrogenase. The efflux in the cytosol of acetyl-CoA, in the form of citrate, is mediated by the citrate carrier (CiC), a protein of the inner mitochondrial membrane. Once in the cytosol, citrate is converted into oxaloacetate and acetyl-CoA by the action of ATP-citrate lyase (ACLY) enzyme. Then, fatty acid synthesis takes place in the cytosol utilizing acetyl-CoA as substrate and the coordinated action of lipogenic enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS).

It is well-known that thyroid hormones have prominent effects on hepatic fatty acid and cholesterol syntheses and metabolism (6, 7). Several enzymatic activities of DNL are increased in response to 3,5,3′-triiodo-L-thyronine (T3) (2, 6–8). T3 can regulate target genes directly by activation of thyroid receptors (9–12) or indirectly through transcription factors (13). Moreover, T3 also mediates non-genomic signaling, which has been described at level of plasma membrane, cytoskeleton, and cytoplasm (14, 15). T3 stimulates the expression of ACC and FAS, upregulating the transcription factor sterol regulatory element binding protein-1 (SREBP-1), the master regulator of lipogenic gene expression (3, 13, 14, 16). T3 also raises the expression of some genes of glucose catabolism to supply acetyl-CoA and NADPH required for DNL (3).

3,5-Diiodo-L-Thyronine (T2) is a natural thyroid hormone derivative once considered only as an inactive product of T3 peripheral metabolism. In recent years T2 has gained a growing interest due to its biological role mainly at the mitochondrial level where it stimulates oxidative capacities and respiration rate both in the liver and skeletal muscle (17–20). Unlike T3, most of the effects of T2 have been reported to occur at the cytosolic rather than at the nuclear level (21). T2 reduces body weight (BW) gain and plasma lipids in high-fat diet (HFD) fed rats (22–25) and decreases hepatic fat accumulation in HFD-fed rats (26, 27) and in a cell model of liver steatosis (28). In most studies so far carried out, the T2 effect was focused on mitochondria where T2 has been indicated to stimulate fatty acid oxidation and respiratory chain activities essentially by short-term mechanisms (20, 21, 29). In this context, T2 effects were more rapid than those of T3 and independent on protein synthesis (20, 21, 29).

On the other hand, it has been demonstrated that T2 when administered to HFD rats, markedly downregulates the gene expression of hepatic SPOT14 (S14), ACC, and FAS (30) and 1 week T2 administration to hypothyroid rats increases oxygen consumption and raises FoF1-ATP synthase activity and expression by a nuclear-mediated mechanism (31).

Given the above, the aim of the present work was to study the long-term effect of T2 administration on the activities and expression of DNL enzymes in a rat model of chemically-induced hypothyroidism. After 1 week of T2 administration to hypothyroid rats, we first followed the rate of total fatty acid synthesis in isolated hepatocytes and then, in rat liver subcellular fractions, the activity and expression of the key enzymes of DNL such as CiC, ACLY, ACC, and FAS. The molecular mechanism of T2 effects on DNL was evaluated by assaying the expression of two lipogenic transcription factors, SREBP-1, and carbohydrate response element binding protein (ChREBP).

EXPERIMENTAL PROCEDURES

Animal Treatment
Male Wistar rats (Harlan, Udine, Italy) (200–250 g) were caged singly in a temperature-controlled room (22 ± 1 °C) with a 12 h light–dark cycle and light on at 8.00 h. Food and water were available ad libitum. Hypothyroidism (Hypo) was induced in rats by the addition of 0.1% propylthiouracil (PTU) in the drinking water for 4 weeks together with a weekly injection of iopanoic acid (IOP) (6 mg/100 g BW) (31). In the last week, some animals from this group were daily injected intraperitoneally with 15 µg T2/100 g BW (Hypo+T2). Euthyroid (Eu) rats received only the vehicle. The animal handling and the experimental design were carried out in agreement with the standards stated in the NIH-Guide for the Care and Use of Laboratory Animals.

Liver and Serum Sample Collection and Biochemical Analyses
Rats were euthanized under anesthesia. The dissected liver was cut into several small pieces and immediately frozen in liquid nitrogen. Serum was obtained from collected rat blood samples. Liver and serum level of cholesterol (SGM Italia, Rome, Italy), triacylglycerols (Futura System, Rome, Italy) and glucose (Futura System, Rome, Italy) were determined by commercial kits.

Preparation of Isolated Hepatocytes and Total Fatty Acid Synthesis Measurement
Rat liver cells were isolated by liver perfusion with collagenase buffer (32). Isolated hepatocytes were suspended in Krebs–Henseleit bicarbonate buffer (KH buffer, pH 7.4) supplemented with 10 mM glucose and 1% (w/v) defatted bovine serum albumin as in (32). Incubations (4–6 mg protein/ml) were carried out in a metabolic shaker at 37°C in 25 mL Erlenmeyer flask under 95% air/5% CO_2 mixture in a final volume of 2 mL. The lipogenic activity was determined by adding [1-^{14}C]acetate (1.56 mCi/mmol) to hepatocyte suspensions and monitoring its incorporation into fatty acids. Reactions were stopped after 1 h with 1 mL of 10 N ethanolic NaOH. Cellular suspensions were transferred to test tubes and, after saponification and acidification with 0.5 mL 7 N HCl, fatty acids were extracted three times with 4 mL of petroleum ether and radioactivity counted (33).

Isolation of Liver Subcellular Fractions
After liver excision, the tissue was homogenized in a buffer containing 250 mM sucrose, 20 mM Tris (pH 7.4) and 1 mM EDTA. Nuclear and mitochondrial fractions were isolated by differential centrifugation. Isolation and purification of nuclei were performed as reported (34). The post-mitochondrial supernatant was ultra-centrifuged at 105,000g per 1 h to obtain the cytosolic fraction. Freshly isolated mitochondria were then used for CiC activity measurements and the cytosolic fraction for the ACC, FAS, ACLY, glucose 6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and isocitrate dehydrogenase (IDH) activity assay. Protein

concentration was determined by the Lowry method with BSA as the reference standard.

Mitochondrial Citrate Carrier Activity Assay

CiC activity was assayed by measuring the rates of malate-[^{14}C] citrate exchange using the inhibitor stop method essentially as described in (35). Briefly, mitochondria were suspended in 100 mM KCl, 20 mM Hepes, 1 mM EGTA, 2 μg/mL rotenone, (pH 7.0), and loaded with L-malate. The rate of exchange [^{14}C] citrate/malate catalyzed by CiC was measured at 9 °C. The transport was started adding to the mitochondrial suspension 0.5 mM [^{14}C]citrate and stopped with 12.5 mM 1,2,3-benzene tricarboxylic acid. Mitochondria were then re-isolated by centrifugation, washed with an isotonic buffer and extracted with 20% HClO$_4$. The labeled citrate, extracted from mitochondria after their osmotic disruption, was counted.

Cytosolic Enzyme Activity Assays

Measurement of ACC and FAS activities was performed essentially as previously described (4). Briefly, ACC activity was measured following the incorporation of [1-^{14}C]acetyl-CoA (20 μCi/mL) into fatty acids in a reaction coupled with that catalyzed by FAS, whereas FAS activity was assayed by measuring the incorporation of labeled acetyl-CoA into fatty acids in the presence of malonyl-CoA. ACC and FAS activities were stopped by 10 M NaOH and samples saponified at 100°C for 45–60 min in capped tubes. After cooling and acidification with 12 M HCl, fatty acids were extracted three times with 4 mL of petroleum ether each time. The combined petroleum ether extracts were evaporated to dryness and residuals dissolved in scintillation fluid and counted for radioactivity.

The activities of ACLY (36), G6PDH (37), ME (38), and IDH (39) were spectrophotometrically determined as reported.

Real-Time RT-PCR and Western Blotting Analyses

Total RNA was isolated by using TRIzol reagent (Invitrogen-Life Technologies Italy, Monza, Italy) according to the manufacturer's instructions. The isolated total RNA was reverse-transcribed by using PrimeScript RT reagent Kit (TaKaRa, Shiga, Japan). Real-time PCR was performed on an ABI 7900 real-time PCR System (Applied Biosystems Italy, Monza, Italy). The primers used for real-time PCR analysis were the following: ACLYFor 5'-GGGAGAAGTTGGGAAGACCA-3'; ACLYRev 5'-GTGCTCCCACT GGCATTAAG-3'; ACCFor 5'-CTTGGAGCAGAGAACCTTCG-3'; ACC Rev 5'-CCTGGATGGTTCTTTGTCCC-3'; CICfor 5'-GCCTCAGCTCC TTGCTCTA-3'; CICrev 5'-ACTACCACTGCCTCTGCCA-3'; FASfor 5'-CTCTGGTGGTGTCTACATTTC-3'; FASrev 5'-GAGCTCTTTC TGCAGGATAG-3'; SREBP1for 5'-AGGAGCCACAATGAAGACCG-3'; SREBP1rev 5'-TAGTCGGTGGATGGGCAG-3'. The gene expression was quantified by normalizing to 18 s.

Western blotting analyses were performed as described before (35). Anti-ACC, anti-SREBP-1, anti ChREBP (Santa Cruz Biotechnology, Santa Cruz, CA), anti-FASN (BD, Biosciences Phar-Mingen, San Diego, CA), anti-CiC (Cell Signaling Technology, Danvers, MA), anti ACLY (BD, Biosciences PharMingen, San Diego, CA), anti-α-Tubulin (Sigma) antibodies were used as primary antibodies. Analysis of ChREBP level was performed on nuclear protein extract, obtained by lysis of purified nuclei in high-salt buffer (20 mM Tris–HCl [pH 7.9], 420 mM NaCl, 10 mM KCl, 0.1 mM Na$_3$VO$_4$, 1 mM EDTA, 1 mM EGTA, 20% [v/v] glycerol).

Statistics

One-way analysis of variance (ANOVA) was used to determine significant differences among groups of animals and two-way ANOVA was adopted to assess differences between groups. When significant values were found ($P < 0.05$), post hoc comparison of the mean was made using Tukey's test. All statistical analyses were performed with the software GraphPad Prism 5. Results were expressed as means ± SEM.

RESULTS

Thyroid State

The serum level of thyroid hormones at the end of animal treatments is reported in Table 1. The Hypo group showed a

TABLE 1 *Serum, BW and liver parameters*

Measured parameters	Eu	Hypo	Hypo + T2
Serum			
FT3 pmol/L	3.42 ± 0.62	1.32 ± 0.35*	1.58 ± 0.44
FT4 μg/dL	2.93 ± 0.33	0.21 ± 0.05*	0.29 ± 0.08*
Phospholipids (mg/dL)	131.1 ± 34.8	122.7 ± 50.8	142.9 ± 50.4*
Cholesterol (mg/dL)	71.1 ± 6.0	83.4 ± 2.6*	81.6 ± 1.5*
Triacylglycerols (mg/dL)	160.7 ± 14.1	68.7 ± 7.0*	95.3 ± 8.9#
Glucose	147.7 ± 15.3	120.8 ± 21.7*	125.5 ± 17.8*
Body			
Final BW (g)	351.7 ± 42.0	244.9 ± 31.1*	249.7 ± 60.1*
Weight gain (g)	108.7 ± 11.1	8.0 ± 0.7*	28.9 ± 2.9#
Liver weight (g)	13.3 ± 2.4	8.7 ± 1.4*	8.4 ± 1.5*
Liver/BW x 100	3.8 ± 0.4	3.6 ± 0.4	3.4 ± 0.5
Liver			
Phospholipids (μg/mg protein)	2.2 ± 0.3	2.0 ± 0.5	2.3 ± 0.6
Cholesterol (μg/mg protein)	5.7 ± 0.9	5.9 ± 2.6	5.6 ± 1.5
Triacylglycerols (μg/mg protein)	21.0 ± 2.4	17.3 ± 5.8	18.0 ± 4.4
Glycogen (μg/mg protein)	22.2 ± 3.7	18.4 ± 3.9	13.4 ± 1.8#

*Values are the mean ± SEM of six different experiments. *P < 0.05 versus Euthyroid (Eu) #P < 0.05 versus Hypothyroid (Hypo). BW = body weight.*
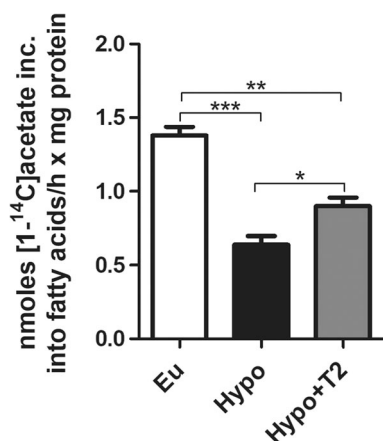
FIG 1 *Total fatty acid synthesis in hepatocyte suspensions. Hepatocytes were obtained by a collagenase perfusion procedure from euthyroid (Eu), hypothyroid (Hypo) and hypothyroid + T2 (Hypo+T2) rats. The total fatty acid synthesis is expressed as the incorporation of [1-^{14}C] acetate into fatty acids/h x mg protein. Values are the means ± SEM of five different experiments. In each experiment, determinations were carried out in triplicate. *P < 0.05; **P < 0.01; ***P < 0.001.*

reduction, with respect to Eu rats, of about 61 and 93% of the serum level of FT3 and FT4, respectively. These values confirmed the effectiveness of the treatment that associates PTU with IOP to obtain a severe hypothyroid status (31). Note that T2 administration to Hypo rats did not significantly affect the FT3 and FT4 serum level.

Serum, Body and Liver Parameters

At the end of the experimental period Hypo rats, when compared to Eu ones, showed an increased serum cholesterol level which was not modified by T2 treatment (Table 1). Triacylglycerol (TAG) level decreased in the serum of Hypo and rose (~40% vs Hypo) after T2 treatment without reaching the Eu value. Glycemia decreased in Hypo versus Eu and did not change after T2 administration.

Hypo had lower gain in body and liver weight with respect to Eu. T2 administration to Hypo animals did not affect the liver weight but induced a significant recovery in BW without reaching the value measured in Eu. However, the liver/BW ratio was not different among the groups (Table 1). No significant change in the hepatic phospholipid, cholesterol, and TAG content was measured among the three groups of rats. Liver level of
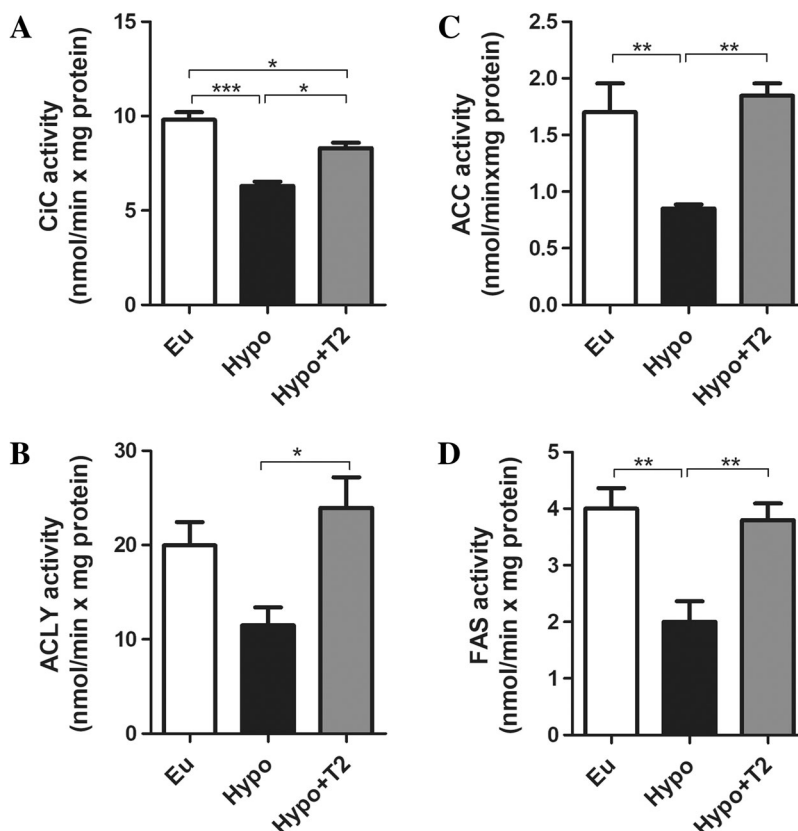
FIG 2 *Hepatic CiC, ACLY, ACC, and FAS activities. Citrate carrier (CiC) (A) activity was measured in the mitochondrial fraction, while ATP-citrate Lyase (ACLY) (B) Acetyl-CoA Carboxylase (ACC) (C) and Fatty Acid Synthase (FAS) (D) in the cytosolic fraction following the procedure reported in the Method's section. Values are the means ± SEM of five different experiments. *P < 0.05; **P < 0.01; ***P < 0.001. Eu = euthyroid; Hypo = hypothyroid; Hypo+T2 = hypothyroid+T2.*

glycogen significantly decreased in Hypo+T2 in comparison with both Hypo and Eu rats (Table 1).

Rate of Total Fatty Acid Synthesis in Hepatocyte Suspensions

T2 action on hepatic fatty acid synthesis was first investigated in isolated hepatocytes obtained from Eu, Hypo, and Hypo+T2 animals. In agreement with previous in vitro experiments where T2 was added to primary cultures of Hypo rats hepatocytes (32), the rate of total fatty acid synthesis from labeled acetate was reduced in hepatocytes from Hypo animals to half of the value observed in Eu (0.64 ± 0.09 nmoles[1-^{14}C]acetate inc./h x mg protein of Hypo vs. 1.42 ± 0.12 nmoles[1-^{14}C]acetate inc./h x mg protein of Eu rats) (Fig. 1). However, when compared with Hypo, a significant (about 40%) increase in the total fatty acid synthesis occurred in Hypo+T2 hepatocytes.

Activity and Expression of CiC, ACLY, ACC, and FAS

We deepened the effect of T2 on fatty acid synthesis by measuring, in rat liver subcellular fractions, the activity and expression of DNL enzymes. The activities of CiC, ACLY, ACC, and FAS were noticeably reduced in Hypo with the greatest effect observed on ACC activity. In all cases, T2 administration increased the enzymatic activities bringing them to values similar to those of Eu (Fig. 2).

In order to investigate the molecular mechanism of T2 effects on DNL, protein and mRNA levels of the above-reported enzymes were next investigated. Protein (Fig. 3) and mRNA (Fig. 4) levels of lipogenic enzymes under consideration changed in parallel to their activities. These findings confirm the existence of a covariance among the four DNL enzymes (5, 40–42).

Activity of NADPH-Producer Enzymes

DNL requires NADPH as a reducing equivalent donor. Classically, cytosolic NADPH is thought to be generated primarily via the oxidative pentose phosphate pathway, ME and IDH (43).

Hypothyroidism decreased, with respect to Eu animals, the activities of G6PDH and ME. T2 administration to Hypo induced a recovery of G6PDH and ME activities which reached values not
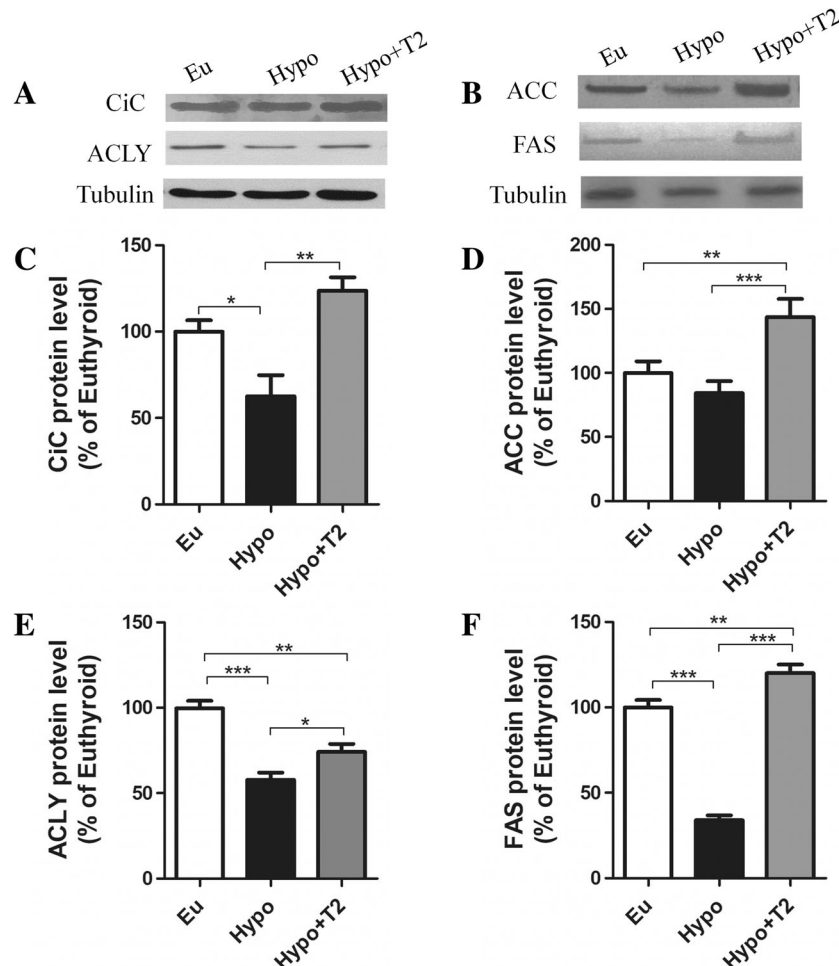
FIG 3 *Hepatic CiC, ACLY, ACC, and FAS protein levels. Representative immunoblot, for Citrate Carrier (CiC) and ATP-Citrate Lyase (ACLY) (A), and for Acetyl-CoA Carboxylase (ACC) and Fatty Acid Synthase (FAS) (B); tubulin was used as loading control. Quantification (mean ± SEM) of immunoblot for CiC, ACLY, ACC, and FAS (C–F). Values are the means ± SEM of five different experiments. *P < 0.05; **P < 0.01; ***P < 0.001. Eu = euthyroid; Hypo = hypothyroid; Hypo+T2 = hypothyroid+T2.*
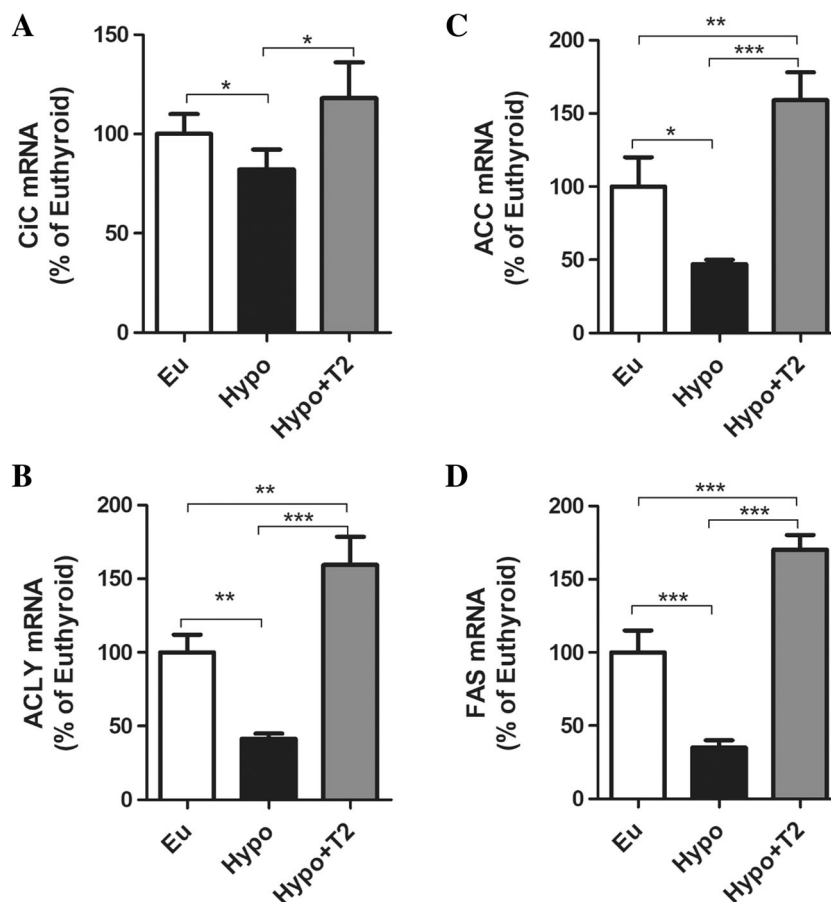
FIG 4 Hepatic CiC, ACLY, ACC, and FAS mRNA amount. Quantification of mRNA amount for citrate carrier (CiC) (A) ATP-citrate Lyase (ACLY) (B) Acetyl-CoA Carboxylase (ACC) (C) and Fatty Acid Synthase (FAS) (D). *P < 0.05; **P < 0.01; ***P < 0.001. Values are the means ± SEM of five different experiments. Eu = euthyroid; Hypo = hypothyroid; Hypo+T2 = hypothyroid+T2.

significantly different from those of Eu (Fig. 5A,B). Neither hypothyroidism nor T2 injection to Hypo rats affected the IDH activity (Fig. 5C).

Mechanism of T2 Effects on DNL

SREBP-1 and ChREBP represent transcription factors regulating hepatic DNL. In Hypo+T2 rats, we found that the mRNA

level of SREBP-1 was significantly reduced with respect to both Eu and Hypo (Fig. 6A). We then evaluated the level of SREBP-1 protein in term of both precursor (p) and nuclear (n) forms. With respect to Hypo animals, both nSREBP-1 and pSREBP-1 levels were significantly increased after T2 administration (Fig. 6B,C). In order to estimate the level of SREBP-1 activation
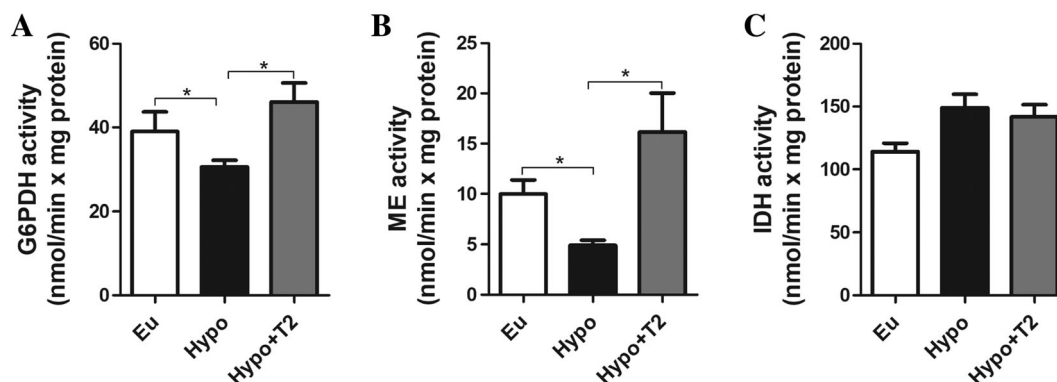
FIG 5 Activities of hepatic NADPH-producer enzymes. G6PDH (A), ME (B), and IDH (C) activities were assayed in the hepatic cytosolic fraction by a spectrophotometric assay following the change in the reduced state of pyridine coenzymes. Values are the means ± SEM of four different determinations. *P < 0.05. Eu = euthyroid; Hypo = hypothyroid; Hypo+T2 = hypothyroid+T2.
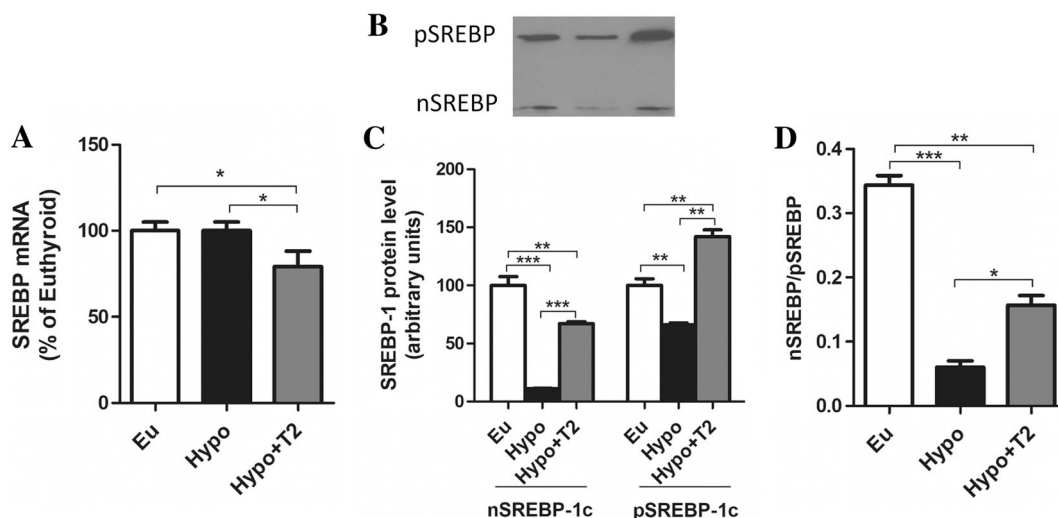
FIG 6 mRNA and protein level of SREBP-1. Hepatic SREBP-1 mRNA amount (A); representative immunoblot for the precursor (p) and mature nuclear (n) SREBP-1 form (B); nuclear (n) and precursor (p) form of SREBP-1 protein level obtained by quantifying Western blotting signals (C) and ratio between the nuclear (n) and the precursor (p) form of SREBP-1 (D). Values are the means ± SEM of six different experiments. *P < 0.05; **P < 0.01; ***P < 0.001.

by T2, we evaluated the nSREBP-1/pSREBP-1 ratio as indicator of the entity of proteolytic cleavage of the precursor in the active mature form (44). Data reported in Fig. 6D indicated that nSREBP-1/pSREBP-1 ratio, reduced in Hypo, was increased by T2 injection still remaining significantly lower than that measured in Eu (Fig. 6D).

Unlike SREBP-1, ChREBP transactivation is controlled by a nuclei-cytoplasmic shuttling mechanism which depends on its phosphorylation status (45). Nuclear levels of ChREBP protein

have been analyzed. Results reported in the Fig. 7 show that in nuclei from Hypo rats the ChREBP level decreased with respect to that observed in Eu. Conversely, when compared to Eu and Hypo, the level of ChREBP was strongly increased in Hypo+T2.

DISCUSSION

T2, a product of T3 peripheral metabolism, is gaining interest due to its rapid effect on energy metabolism without inducing unfavorable thyrotoxic effects (23). T2 has been demonstrated to reduce BW gain and liver steatosis in HFD-fed rats (24, 25, 27) and lipid accumulation in a model of hepatosteatosis (28). The anti-lipidemic effect of T2 has been mainly ascribed to a concomitant activation of fatty acid oxidation and downregulation of lipogenesis (46).

Although T2 effects on hepatic lipid catabolism have been well elucidated (17, 18, 20, 22, 23, 26–28), very little is known about its possible action on DNL and on lipogenic gene expression in a hypothyroid state (32). A very recent work demonstrated that both T3 and T2 reduced fat accumulation in HFD rats influencing DNL but with a different molecular mechanism (30).

In the present study, we followed in Hypo rats the effect on DNL of T2 at a dose (15 µg/100 g BW for 1 week) that was already demonstrated to be metabolically effective (31, 47). Notably, considering serum thyroid hormone levels (Table 1), it can be inferred that the observed T2 effects on DNL may be ascribed to a direct action of the diiodothyronine rather than to an indirect one due to its conversion to T3.

Moreover, in order to exclude a possible systemic interference, we assayed lipogenesis in an in vitro system, by isolating hepatic cells from the three groups of rats. The results of these experiments demonstrated that T2 is able to increase the total fatty acid synthesis in a cell autonomous manner.
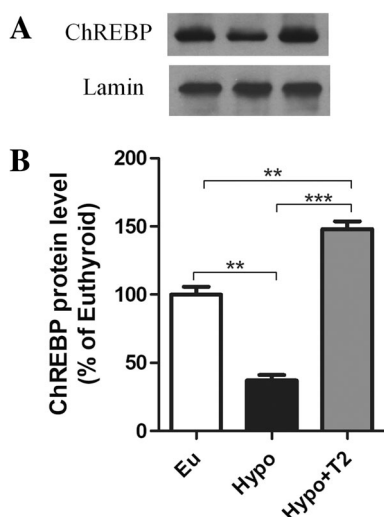
FIG 7 Protein level of ChREBP. (A) Representative immunoblot of the nuclear ChREBP protein obtained by Western blotting. (B) Quantitative analysis of ChREBP protein level obtained by quantifying Western blotting signals. Values are the means ± SEM of three different experiments. ***P < 0.001; **P < 0.01. Eu = euthyroid; Hypo = hypothyroid; Hypo+T2 = hypothyroid+T2.

Our results demonstrate that T2, when injected to Hypo rats for 1 week, is able to increase both the mRNA amount and the protein level of the cytosolic DNL enzymes ACLY, ACC, and FAS and to induce a rescue of their activities near to values of Eu. Moreover, T2 increases the activity and the expression of CiC, a protein of the inner mitochondrial membrane strictly correlated to DNL. CiC, in the form of citrate, transports from the mitochondria to the cytosol acetyl-CoA, starter molecule of fatty acid synthesis. In addition, citrate in the cytosol may allosterically activate ACC, the key regulatory enzyme of DNL. Our data, besides confirming a covariance of these lipogenic activities (4, 5, 40–42) indicate that T2, similarly to T3, stimulates DNL of Hypo rats by a long-term mechanism.

CiC expression was demonstrated to be inhibited in Hypo both at transcriptional and posttranscriptional levels (2, 34). Moreover, SREBP-1, the master regulator of lipogenic gene expression, has been reported to play a role in the transcriptional regulation of CiC, as well as of ACC and FAS (3). Here we show that the mRNA level of SREBP-1 is significantly reduced by T2 administration to Hypo animals (Fig. 6).

SREBPs family includes SREBP-1a, SREBP-1c, and SREBP-2, transcription factors involved in the control of lipid homeostasis through the regulation of several genes (48). SREBPs are synthesized as inactive precursors (pSREBPs) bound to the endoplasmic reticulum, where their regulatory domain interacts with SREBP cleavage-activating protein (SCAP) which represents a sensor for membrane cholesterol level. When an activation signal occurs, the SREBP-SCAP complex translocates from the endoplasmic reticulum to the Golgi apparatus, where a two-step proteolytic cleavage releases the N-terminal half of SREBPs nuclear form, allowing its entry into the nucleus where gene transcription regulation occurs (49).

We found that T2 caused, in Hypo rats, an increase in the level of both precursor pSREBP-1 and mature nSREBP-1 forms. The value of nSREBP-1/pSREBP-1 ratio was also increased after T2 treatment of Hypo animals. These data indicate that T2 is able to increase the proteolytic cleavage of pSREBP-1 thus raising the level of the active nSREBP-1 form. On this basis, it is reasonable to speculate that T2, by activating translational and posttranslational mechanisms, enhances SREBP-1 mRNA translation and activates SREBP-1 proteolytic cleavage.

ChREBP is a basic helix–loop helix/leucine zipper transcription factor and is involved in mediating glucose-responsive gene activation. This transcription factor is implicated in the activation of liver-type pyruvate kinase (L-PK), S14, FAS, and ACC, and thus regulates the glucose and lipid metabolism. A phosphorylation/dephosphorylation mechanism controls the ChREBP transactivation. During fasting, ChREBP is retained in the cytosol, inactivated by protein kinase A-mediated phosphorylation. After feeding, xylulose-5-phosphate, produced by glucose metabolism through the pentose phosphate pathway, activates protein phosphatase 2A, which dephosphorylates ChREBP. Dephosphorylated ChREBP moves in the nucleus and induces the expression of the target genes (45).

In agreement with the results observed in liver from Hypo mice (50) we found that the nuclear content of ChREBP was reduced in Hypo rats. After T2 administration, ChREBP level strongly increased with respect to both Eu and Hypo rats, suggesting a prominent role of this transcription factor in the lipogenic effect of T2 on Hypo rats.

Moreover, according to (51) in Hypo rats we found that T2 increased the activity of G6PDH and ME, main responsible for the cytosolic provision of NADPH for reductive reactions in DNL. These data further strengthen our observations regarding the pro-lipogenic effect of T2 in hypothyroid rats. At this point it must be underlined that G6PDH is the rate-limiting enzyme of the pentose phosphate pathway by which is produced xilulose-5-phosphate, the activator of ChREBP. This finding further supports the involvement of ChREBP in the activation of DNL by T2.

SREBP-1 has been demonstrated to have a positive effect on G6PDH expression (52). Activation of ME expression by SREBP-1 has been also described (52). Thus, it can be hypothesized that T2 injection to Hypo rats, by increasing nSREBP-1 level, can be also responsible for the increased G6PDH and ME activity. The fact that IDH activity was not significantly affected by T2 injection demonstrated that T2 specifically affects the above reported lipogenic activities. To note that a similar behaviour has been also reported in animals treated with T3 (53–56).

In agreement with (57–60), we found that, when compared to Eu, Hypo rats showed increased serum cholesterol and decreased TAG level. T2 administration to Hypo tended to restore serum TAG amount but did not affect the level of cholesterol. Moreover, T2 administration significantly decreased the hepatic glycogen content with respect to both Eu and Hypo rats. Considering that CiC and ACLY activities represent a bridge between carbohydrate catabolism and lipid synthesis, their hepatic activation after T2 administration, increasing glucose utilization, can contribute to the lower level of hepatic glycogen we measured after T2 administration (see Table 1). Accordingly, a T2-stimulated glucose utilization has been also recently demonstrated in cardiomyoblasts (61).

Our results look different from those previously reported in the literature where T2 has been shown to stimulate catabolic pathways (17, 18, 20, 22, 23, 26–28) while inhibiting fatty acid synthesis (30). We think that such discrepancy can be ascribed, at least in part, to the different experimental conditions and animal models used. Indeed, in most studies, T2 effects were evidenced after a short-term period and mainly in HFD-fed rats (26, 27, 30) or in a cell model of liver steatosis (28). In these conditions, a marked lipid accumulation was present in the liver which was significantly reduced upon T2 injection to rats (30). Vice versa, in the present study, T2 was administered for 1 week to Hypo rats where, as previously demonstrated, fatty acid synthesis is greatly reduced (2, 34).

Overall, our results represent the first evidence that enzyme activities of DNL are significantly stimulated by T2 treatment in Hypo rats. In this context, we showed that SREBP-1 and ChREBP dependent mechanisms are involved in the transcriptional activation of DNL genes by T2. Obviously,

further studies are needed to elucidate the overall mechanism of T2 effect of DNL.

CONFLICT OF INTEREST

All the authors declare that they have no conflict of interest.

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